REMARKS

This Amendment is responsive to the Office Action mailed on October 18, 2007. With this Amendment, claims 5 and 7 are cancelled, and claims 1, 6, and 8 amended. Claims 1-4, 6, 8-12, and 25 are pending and under consideration with this Amendment.

Claim Rejections – 35 USC § 102

The Office Action rejects claims 1-4 and 25 under 35 U.S.C. § 102(b) as allegedly being anticipated by Xia *et al.* ("siRNA-mediated gene silencing in vitro and in vivo," Nat. Biotech. 20:1006-1010 (2002)). Specifically, the Office Action rejects claims 1-4 based on the alleged recital in the cited publication of a construct that expresses a 21-bp siRNA with a poly(A) cassette that forms a hairpin structure, wherein the hairpin comprises a sequence that is complementary to enhanced green fluorescent protein and is placed under the control of the CMV promoter. The Office Action rejects claim 25 on the basis that the cited publication allegedly teaches transfection of the construct into mammalian cells.

In response, and without agreeing or acquiescing to the rejection, Applicants note that claim 1 has been amended to recite elements present in claims 5 (now element d of claim 1) and 7 (now element e of claim 1). As claims 5 and 7 were not rejected over the cited art, Applicants respectfully submit that claim 1 is not rejectable over the cited art.

The Office Action also rejects claim 25 in light of the transfection disclosure in Xia *et al.* Claim 25 depends from claim 1 and is not anticipated by the cited art for at least the same reasons as claim.

Applicants respectfully request withdrawal of the 35 USC § 102 rejections.

Claim Rejections – 35 USC § 103

The Office Action rejects claims 1-4 and 7-8 under 35 USC § 103(a) as being unpatentable over Xia *et al.* in view of Perkins *et al.* (US2003/0119104 A1) and Yonaha *et al.* (presumably Yonaha *et al.*, Molecular Cell, Vol. 3, pages 593-600 (1999), though not stated in the Office Action).

Initially, Applicants note that the Examiner fails to indicate which Yonaha *et al.* document is cited, though she refers to Yonaha *et al.*, Molecular Cell, Vol. 3, pages 593-600 (1995). Applicants respectfully submit that the reference to a 1995 publication date appears to be in error; the publication date should be 1999. A copy of the front page of the cited article is attached for the Examiner's convenience, and Applicants respectfully request that the Examiner clarify the record with regard to this citation.

In response, and without agreeing or acquiescing to the rejection, Applicants note that claim 1 has been amended to recite elements present in claim 5 (now element d of claim 1). As claim 5 was not rejected over the cited art, Applicants respectfully submit that claim 1 is not rejectable over the cited art as combined by the Action.

The Office Action rejects claims 1-6 and 25 under 35 USC § 103(a) as being unpatentable over Xia *et al.* in view of Huang *et al.*

In response, and without agreeing or acquiescing to the rejection, Applicants note that claim 1 has been amended to recite elements present in claim 7 (now element e of claim 1). As claim 7 was not rejected over the cited art, Applicants respectfully submit that claim 1 is not rejectable over the cited art as combined by the Action.

Finally, the Office Action rejects claims 1-4, 10-12, and 25 under 35 USC § 103(a) as being unpatentable over Xia *et al.* in view of Nomura *et al.* and GenBank accession no. AF435852.

In response, and without agreeing or acquiescing to the rejection, Applicants note that claim 1 has been amended to recite elements present in claims 5 (now element d of claim 1) and 7 (now element e of claim 1). As claims 5 and 7 were not rejected over the cited art, Applicants respectfully submit that claim 1 is not rejectable over the cited art as combined by the Action.

Applicants respectfully request withdrawal of the 35 USC § 103(a) rejections.

Claim Rejections – 35 USC § 112, second paragraph

The Office Action rejects claims 5-6 under 35 USC 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, claim 5 is rejected for not providing a proper antecedent basis for the limitation "RNA located upstream" of the nucleotide (a) and (c). Claim 6 is rejected for being vague and indefinite as to the phrase "ribozyme site."

In response, Applicants note that claim 5 has been canceled and claim 1 has been amended to include the claim language originally in claim 5, rendering moot the rejection as applied to claim 5. While disagreeing that claim 6 was indefinite, Applicants note that claim 6 has been amended to state "a ribozyme site which is a sequence capable of being autocatalytically cleaved by the ribozyme activity." Applicants submit that the amendment to claim 6 renders the claim even more definite.

Claim Rejections – 35 USC § 112, first paragraph

The Office Action rejects claims 1-12 and 25 under 35 USC 112, first paragraph, for failing to comply with the written description requirement. Applicants respectfully disagree with the rejection.

Initially, Applicants respectfully note that the Office Action rejects all of the pending claims as failing to comply with the written description requirement. In the rejection, the Examiner states that the specification does disclose "SEQ ID NO:1, which is a 540 base pair region of a Ski gene" (page 9, lines 9-10), seemingly suggesting that one particular embodiment is disclosed. Yet, the Action rejects claim 12, which specifically recites, "a part of the target gene is a 540 bp 5'-region of the Ski gene," which is the embodiment the Examiner appears to indicate is fully disclosed. Applicants respectfully request clarification from the Office as to what it believes is fully described, so that an appropriate response may be made. Indeed, Applicants respectfully submit that the Office has failed to set forth a reasoned explanation as to why claims 12, and any other dependent claims, are not fully described.

Applicants also respectfully traverse the rejection for the following reasons. As noted above, claim 1 has been amended to recite elements from claims 5 and 7. Applicants also note that claim 1 has been amended to recite that "the ds-RNA is capable of inducing RNA interference." Thus, the claim is limited to ds-RNA expression vectors that are capable of inducing RNA interference. A person skilled in the art can confirm whether ds-RNA of interest can induce RNA interference using known assays. Thus, a person of skill in the art can readily determine what target sequence in which target gene should be used in the vector of the present invention. Applicants should not be required

to disclose all possible target sequences usable in the presently claimed invention – to do so would unnecessarily add to the disclosure, as such information is known or readily determinable from the art.

To the Action's comment that "the disclosure does not provide an adequate description of a MAZ domain, or further wherein which part of said MAZ domain would function as a pause site," Applicants respectfully note that Yonaha *et al.* (cited by the Action) describes such MAZ domains, including a sequence to which MAZ binds and which can pause polymerase II. Thus, a person of skill in the art can readily determine which sequence can act as a MAZ domain by means of assays such as those described in Yonaha *et al.* Again, Applicants should not be required to disclose information in the specification that is readily available to persons of skill in the art.

In view of the foregoing remarks and amendments, Applicants respectfully request withdrawal of the rejection under 35 U.S.C. § 112, first paragraph.

Any comments or questions concerning this application can be directed to the undersigned at the telephone number given below.

Respectfully submitted,
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Specific Transcriptional Pausing Activates Polyadenylation in a Coupled In Vitro System

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Summary

We have developed a coupled in vitro transcription–polyadenylation system to investigate RNA polymerase II (Pol II) termination, which depends on active polyadenylation of the nascent RNA. Specific G-rich sequences originally identified as binding sites for the transcription factor MAZ both pause Pol II and activate polyadenylation of an upstream poly(A) signal. They do not affect polyadenylation efficiency in an uncoupled cleavage assay. In contrast, pausing of Pol II elongation induced by a high-affinity DNA-binding protein does not activate polyadenylation, indicating that G-rich *MAZ* sequences have a specific effect on polyadenylation. They also promote intrinsic pausing of purified Pol II, indicating a general role in the modulation of cotranscriptional RNA processing events.

Introduction

We have previously investigated termination of Pol II transcription between the closely linked human complement genes *C2* and factor B, which are separated by only 421 bp. Initially it was demonstrated by using a combination of nuclear runoff and poly(A) signal competition assays that transcriptional termination occurs within a 160 bp sequence, located just downstream of the *C2* poly(A) signal. Furthermore, a specific sequence (GGGGGAGGGGG) within this region is required for Pol II termination and was shown to bind the zinc finger protein MAZ (Ashfield et al., 1991, 1994), which also binds to a site within the c-*myc P2* promoter (Bossone et al., 1992). *MAZ* sites are also present between the closely spaced human genes *g11–C4* and within an intron of the mouse *IgM-D* gene, lying within sequences that have termination activity (Ashfield et al., 1994).

To investigate the biochemical mechanism of Pol II transcriptional termination, it is necessary to develop an in vitro assay system. This approach has been successfully applied to elucidate the mechanism by which elongation of Pol I is blocked by the termination factor TTF I (Kuhn et al., 1990). Together with a release factor, TTF I mediates termination of transcription (Jansa et al., 1998). An extra complexity in developing an in vitro system for Pol II transcriptional termination is its known dependence on the cleavage/polyadenylation reaction (Proudfoot, 1989; Dye and Proudfoot, 1999). We have therefore developed a coupled in vitro transcription–polyadenylation system. Using this system, we demonstrate that the presence of specific G-rich sequences positioned downstream of a strong poly(A) signal elicits transcriptional pausing, leading to the activation of polyadenylation. These results demonstrate a mechanistic coupling between the specific pausing of elongating Pol II and the activation of polyadenylation.

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Results

An in vitro system in which Pol II transcription and polyadenylation occur as coupled reactions was previously reported using the mouse adenosine deaminase (*ADA*) promoter (Mifflin and Kellems, 1991). However, polyadenylation in this system was inefficient due to the relatively low transcription activity of the *ADA* promoter. Therefore, we have employed the adenovirus major late promoter (MLP), which has stronger transcriptional activity. In vitro cleavage/polyadenylation reactions with synthetic RNA substrates require low $MgCl_2$ concentrations with 1 mM as the optimal condition (Moore and Sharp, 1985). However, our coupled system requires 4 mM $MgCl_2$ for the transcription-dependent polyadenylation reaction, even though these conditions inhibit the processing of exogenous RNA in nuclear extract.

G-Rich *MAZ* Sites Activate Polyadenylation in a Coupled System

Figure 1a shows transcripts produced from DNA templates containing MLP followed by a synthetic poly(A) site (SPA; Levitt et al., 1989) or a mutant form of SPA lacking the AATAAA sequence. The wild-type SPA template generates both readthrough and polyadenylated RNA products (lanes 2–5). These run as a characteristic smear above the readthrough band (corresponding to the EcoRI site used to cleave the DNA template). The mutant SPA template gives no detectable poly(A) product (lanes 6 and 7). We have previously shown that four copies of the *MAZ* DNA-binding site arranged in tandem function as a potent transcriptional pause site using a poly(A) site competition assay (Ashfield et al., 1994). We therefore inserted either four wild-type or mutant *MAZ* sites just downstream of the SPA and carried out the coupled in vitro transcription assay (Figure 1b). Surprisingly, the presence of the four wild-type *MAZ* sites strongly activates the polyadenylation activity (compare lanes 2–5 with 6–9). The apparent increase in overall transcription at later time points as shown in lane 5 as compared to lanes 9–11 is likely to be due to the increased stability of the polyadenylated RNA compared to readthrough product rather than an overall increase in transcription efficiency (see Figure 3). In this experiment, the DNA template was cleaved further downstream so that the polyadenylated product now runs ahead of the readthrough position. This product was shown to be polyadenylated by oligo(dT) cellulose selection (data not shown, but see Figure 2c). As expected, a smaller RNA species corresponding to cleaved but unpolyadenylated